

Scheme 1 Solid Phase Protein Synthesis
Native Chemical Ligations in an N- to C- Terminal Direction

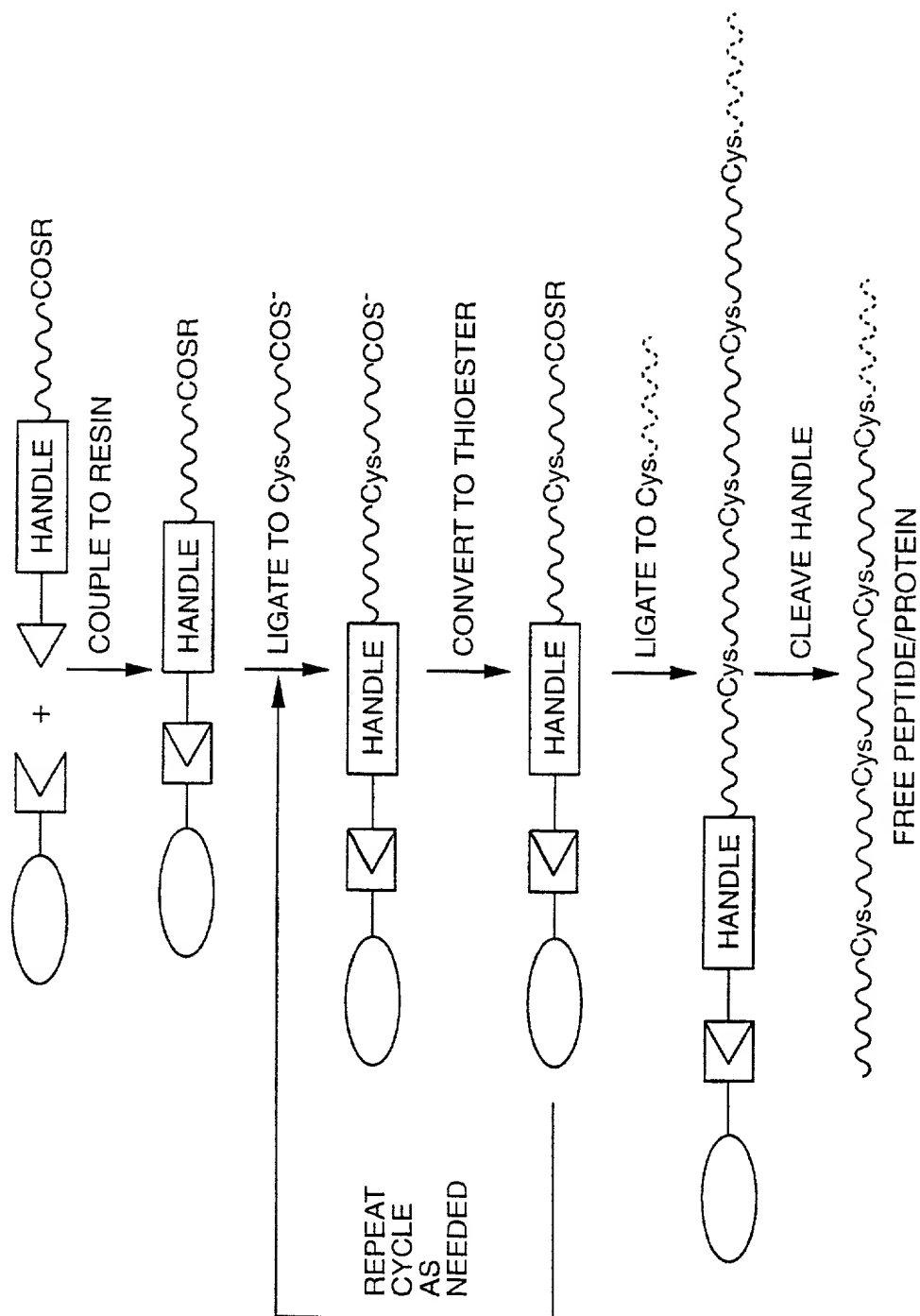


FIG. 1

IN THE ABSENCE OF A THIOESTER PEPTIDE
H - CGFRVREFGDNTA - *COSH* MW=1487.6
 6M GU•HCL, 0.1M NaPi, 0.5% THIOPHENOL, ROOM TEMPERATURE,
 OVERNIGHT

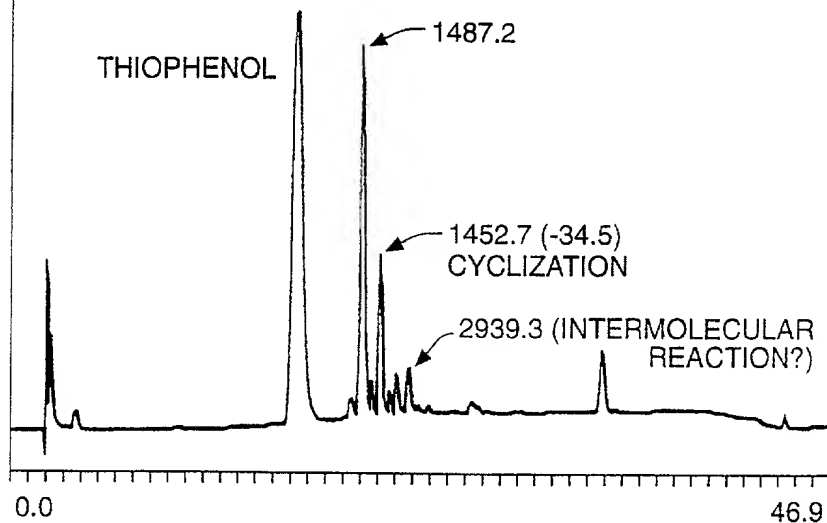


FIG._2A

IN THE PRESENCE OF A THIOESTER PEPTIDE
H - CGFRVREFGDNTA - *COSH* MW=1487.6 + *H* - DSVISLSGDH - *SPAL*
 MW=1230.2 MW OF LIGATION PRODUCT = 2498.7

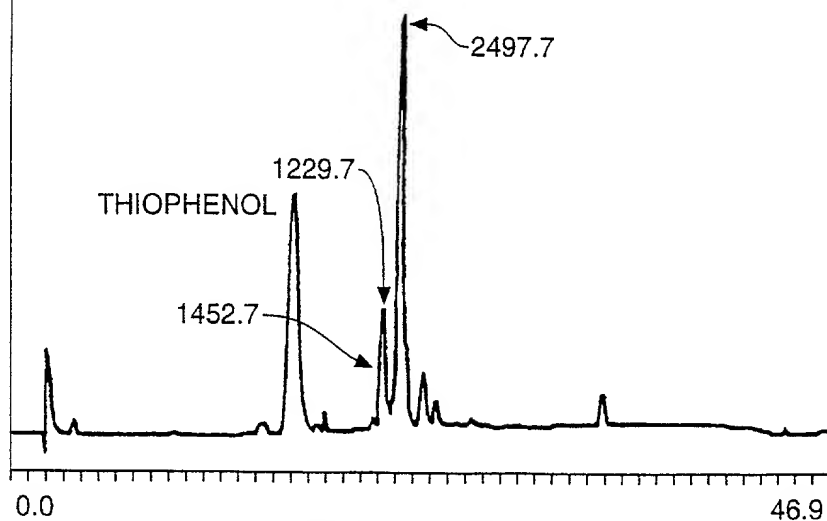
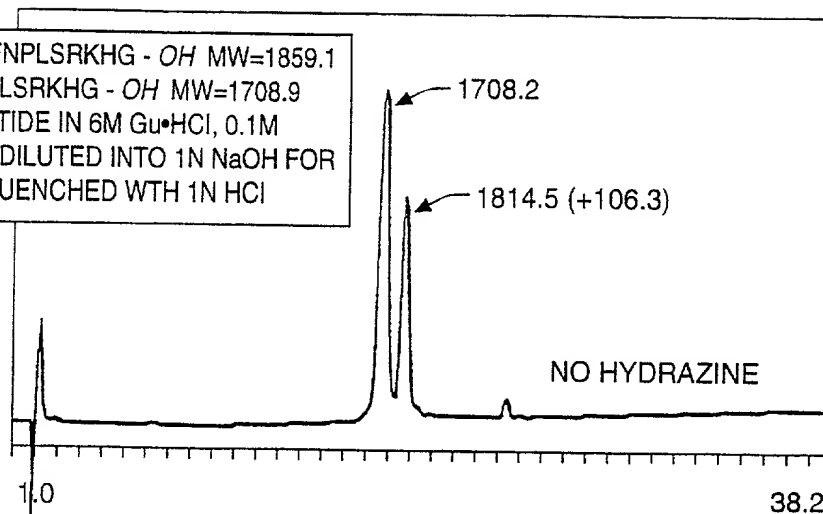


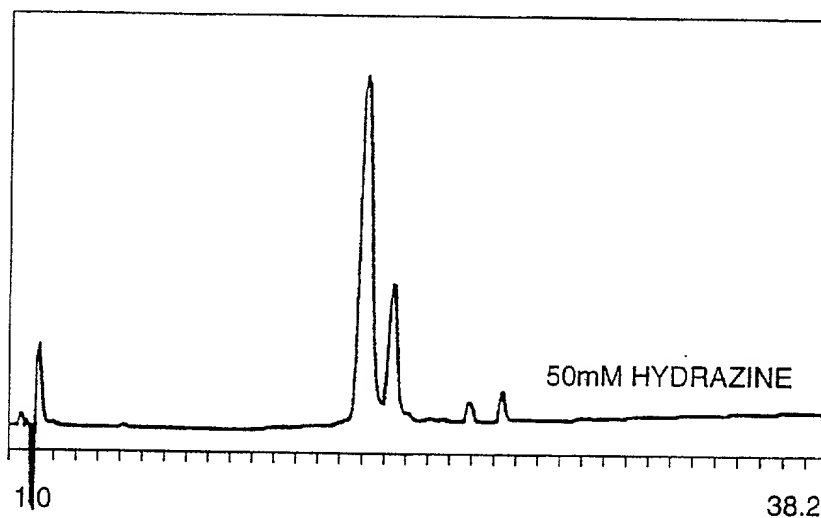
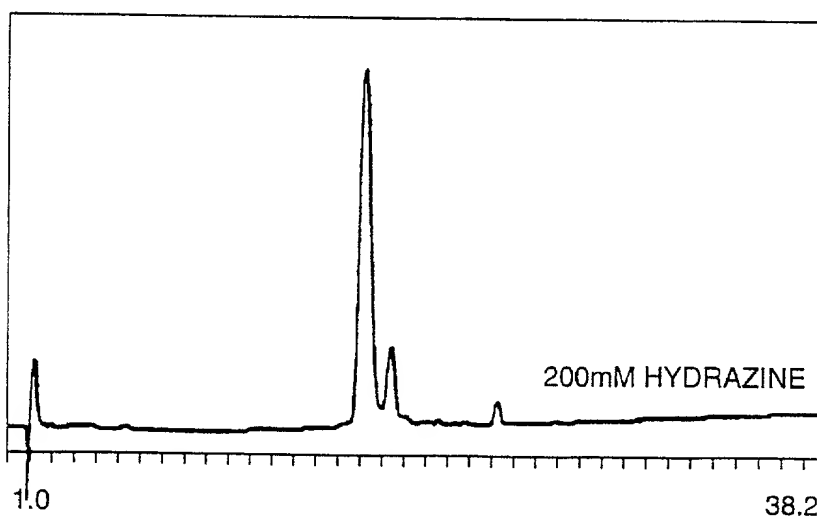
FIG._2B

Cys +COSR Stability Under Ligation Conditions

MSC - CTSAGPHFNPLSRKHG - OH MW=1859.1
 H - CTSAGPHFNPLSRKHG - OH MW=1708.9
 ALIQUOT OF PEPTIDE IN 6M Gu•HCl, 0.1M
 NaPi, pH 7.5 WAS DILUTED INTO 1N NaOH FOR
 TWO MINUTES, QUENCHED WTH 1N HCl

FIG._3A

MSC
 Removal
 Experiments

FIG._3B**FIG._3C**

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Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH
MW=4022.4

H - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH
MW=3745.1

ALIUOT OF PEPTIDE IN 6M Gu•HCl, 0.1M NaAc, pH 4.6 WAS DILUTED
INTO 6M Gu•HCl, 0.1M NaAc, pH 14 FOR TWO MINUTES, QUENCHED WITH
6M Gu•HCl, 0.1M NaAc, pH2.0

MSC Removal
Experiments
(Cont'd)

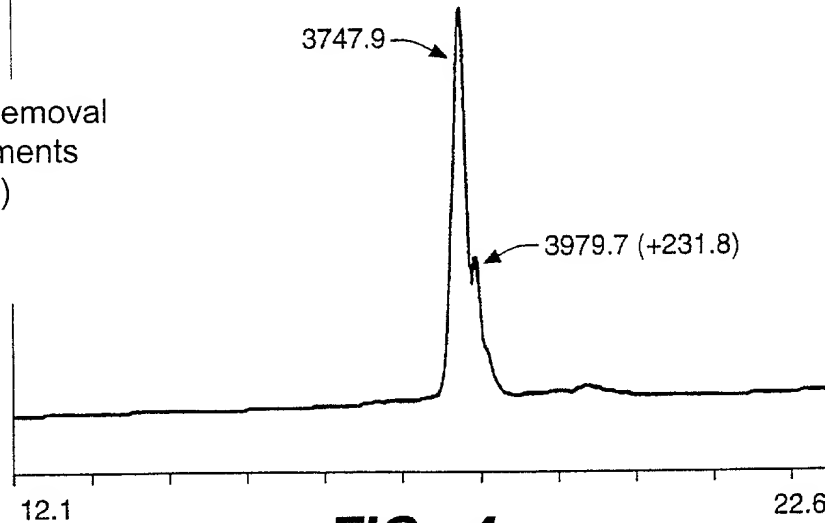


FIG. 4

1 21 47
TLQKKIEEIAAKYKHSVVKCCYDGACVNNDETCEQRAARISLGPCKIKAFTECC
VVASQLRANISHKDMQLGR
74

Synthesis of C5a by Solid
Phase Chemical Ligations
in the N- to C-Terminal
Direction

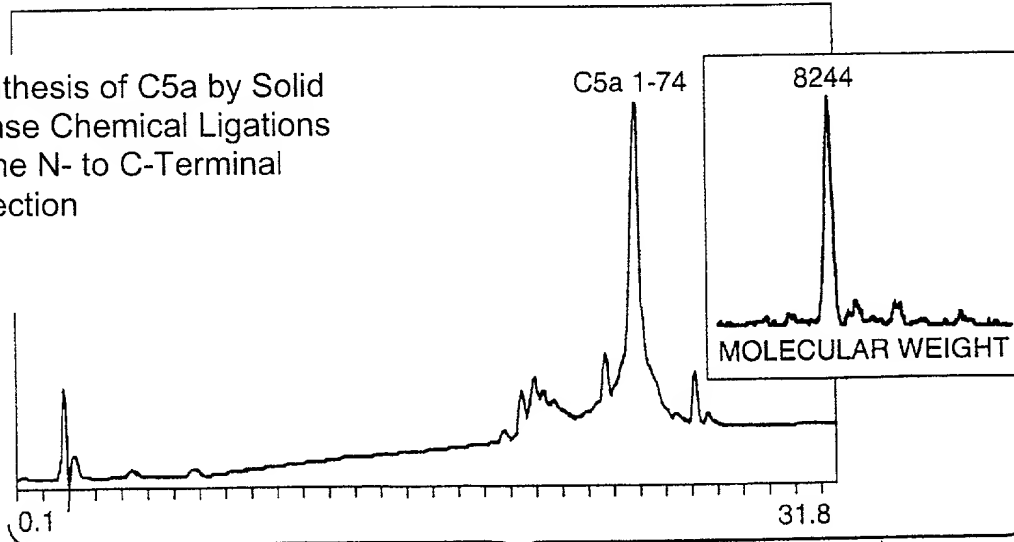
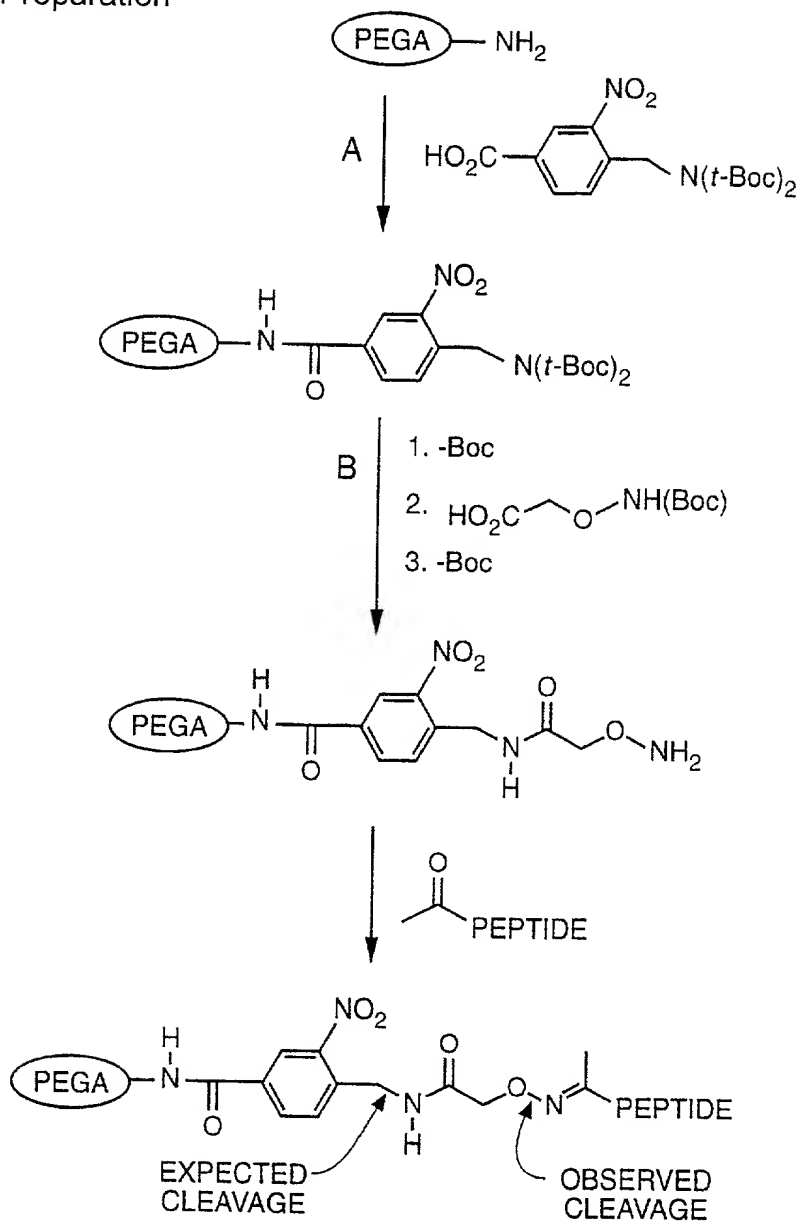


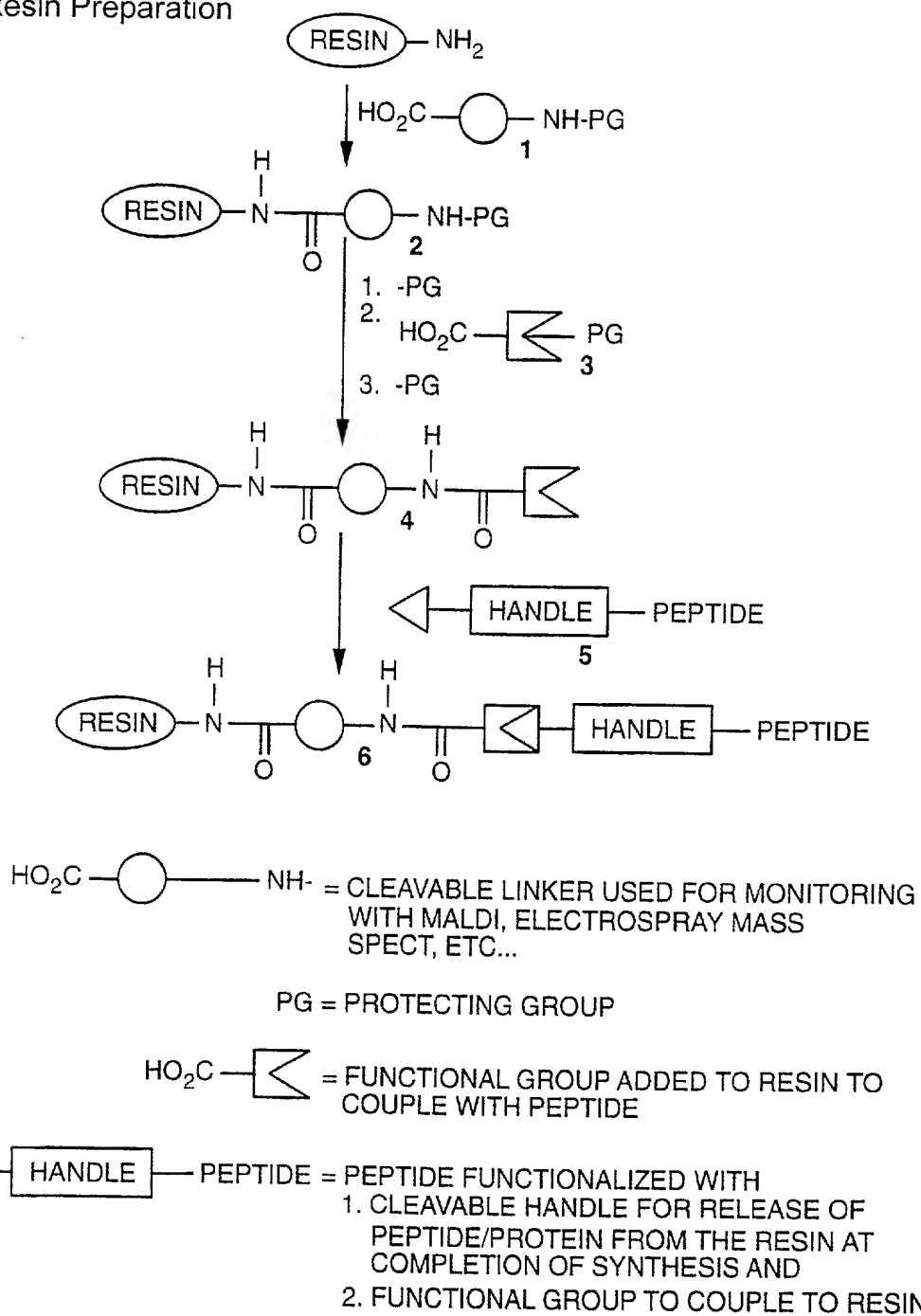
FIG. 26

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Resin Preparation

**FIG. 5A**

Resin Preparation

**FIG. 5B**

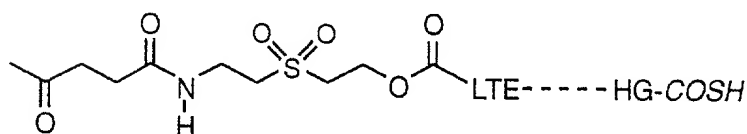
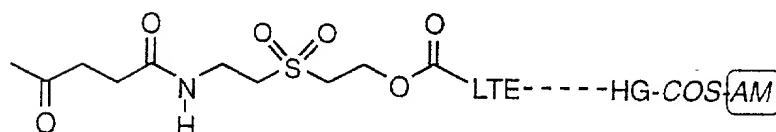
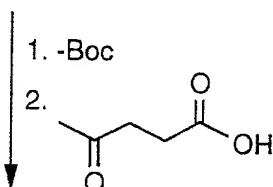
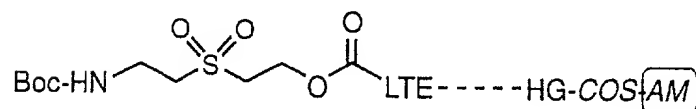
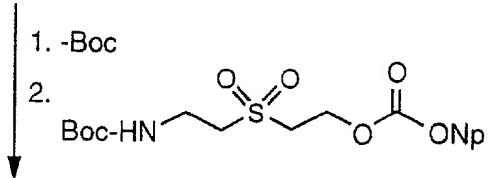
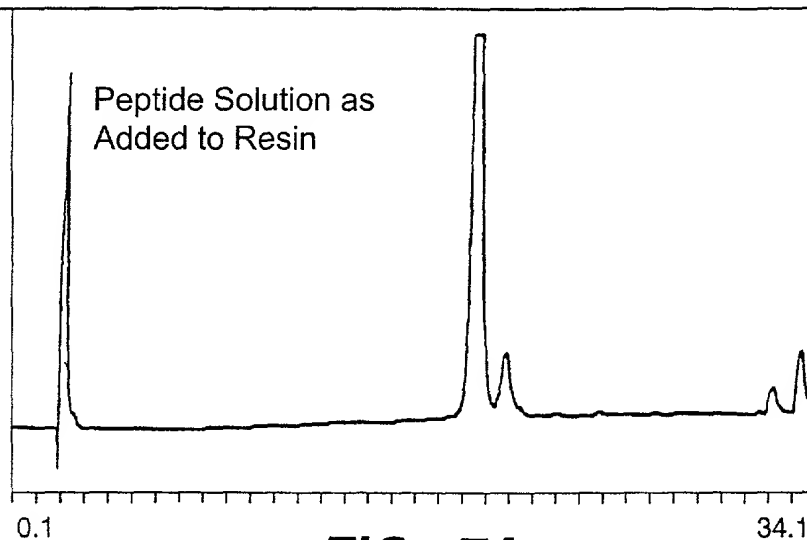


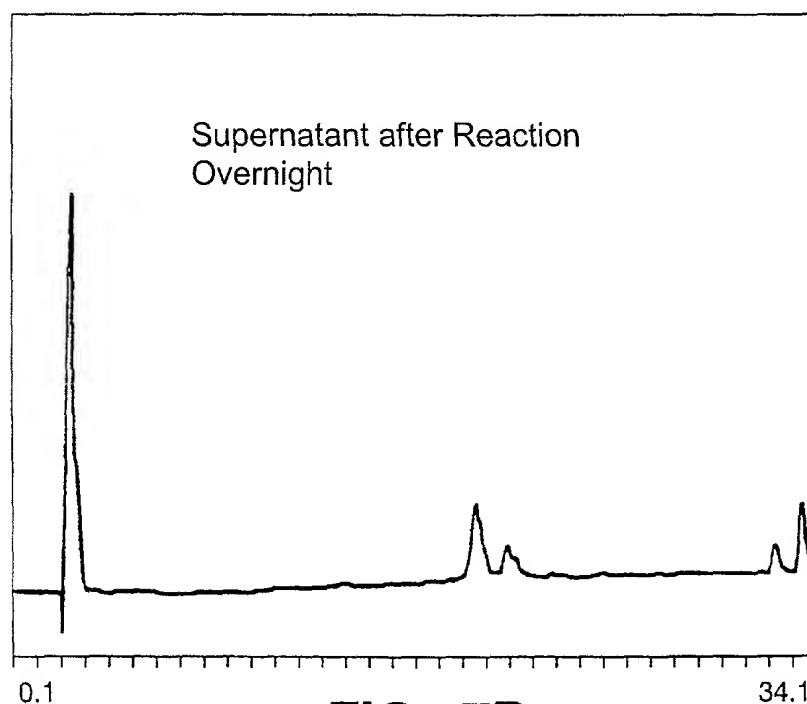
FIG. 6

Derivatization of Segment 1
(N-terminal)

Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH (1)
+ Resin - PCL - ONH₂
↓ 1. pH 4.6, 6M Gu•HCl, 0.1 ACETATE
Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH (1)

**FIG._7A**

Polymer-Supported Ligation on PEGA

**FIG._7B**

Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH (1)
+ Resin - PCL - ONH₂
↓ 1. pH 4.6, 6M Gu•HCl, 0.1 ACETATE
Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH (1)

Polymer-Supported Ligation on ISCO

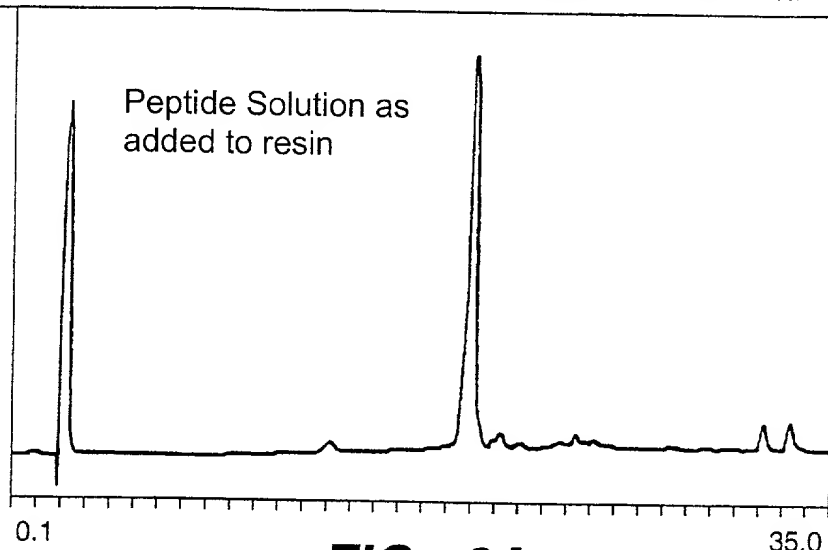


FIG._8A

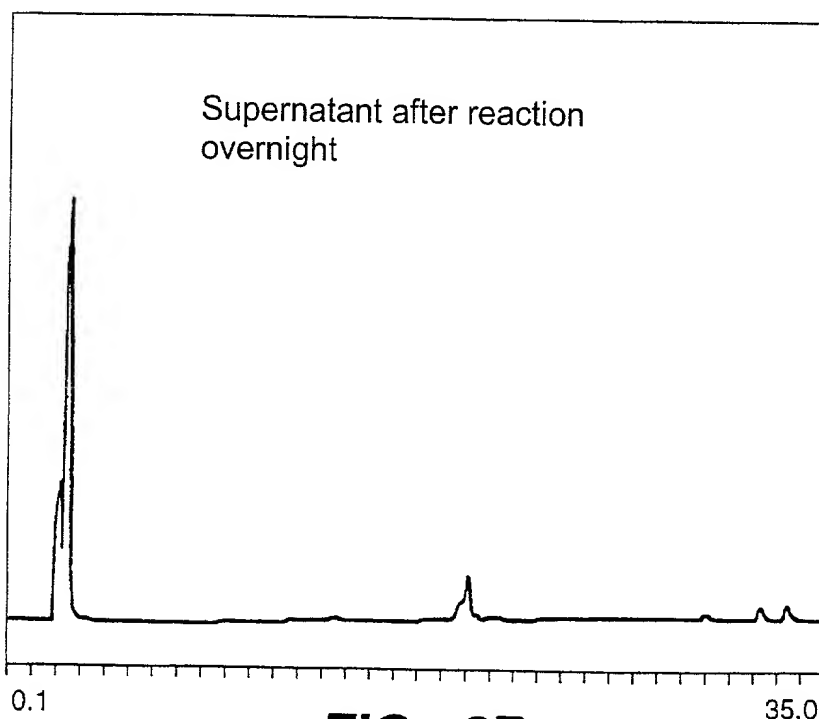


FIG._8B

Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH (1)
+ Resin - PCL - ONH₂

↓ 1. pH 4.6, 6M Gu•HCl, 0.1 ACETATE

Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSH (1)
MALDI MASS = 4022, BASE CLEAVAGE MASS = 3745

Polymer-Supported
Ligation on
ISCO

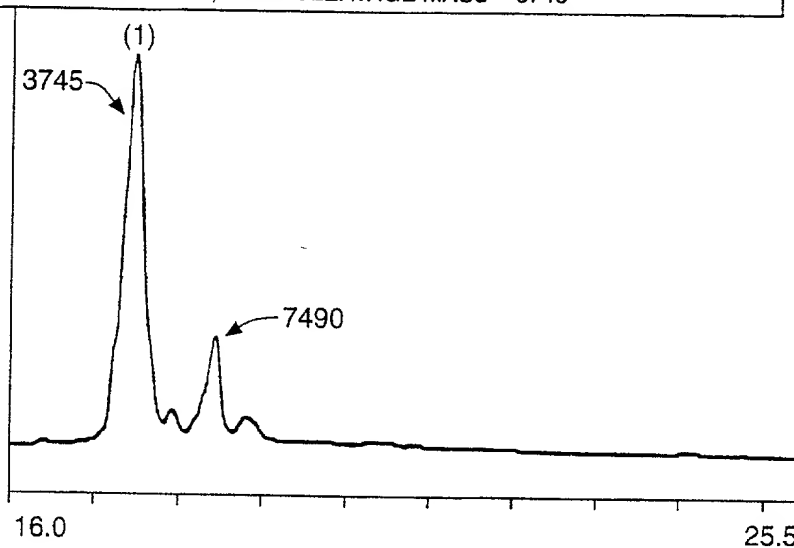


FIG._9A

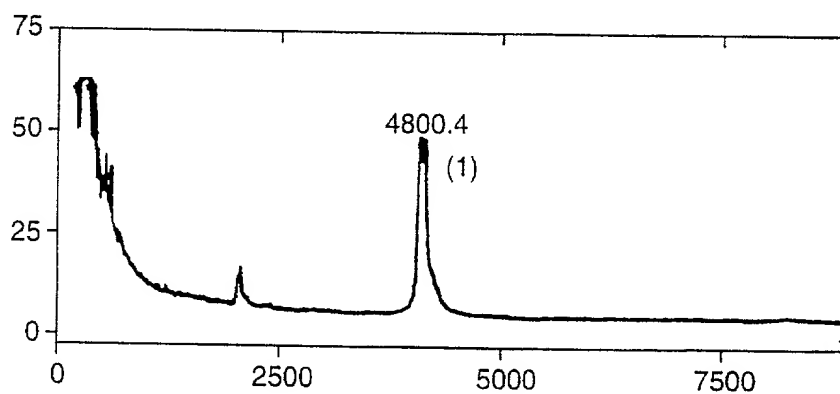


FIG._9B

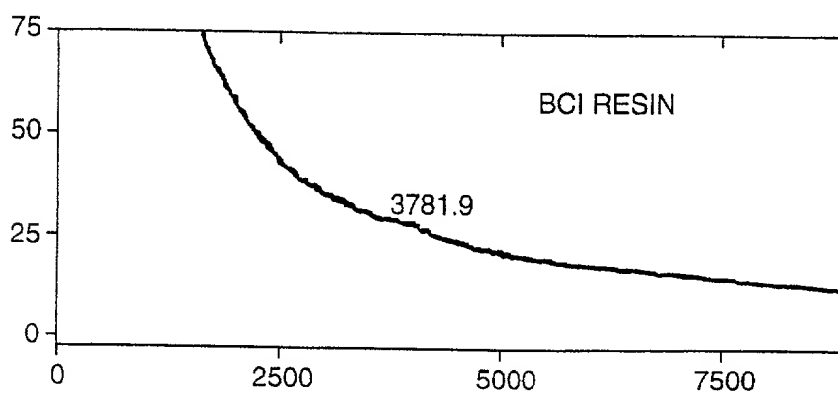


FIG._9C

Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - COSAc (1)
 MALDI MASS = 4080, BASE CLEAVAGE MASS = 3729
 + H - CGFRVREFGDNTA - COSH (2)

↓ 3. pH 7.5, 6M Gu•HCl, 0.1M PHOSPHATE, 0.5% THIOPHENOL

Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHGCGFRVREF -
 GDNTA - COSH (1+2)
 MALDI MASS = 5476, BASE CLEAVAGE MASS = 5199

Polymer-
Supported
Ligation on
ISCO

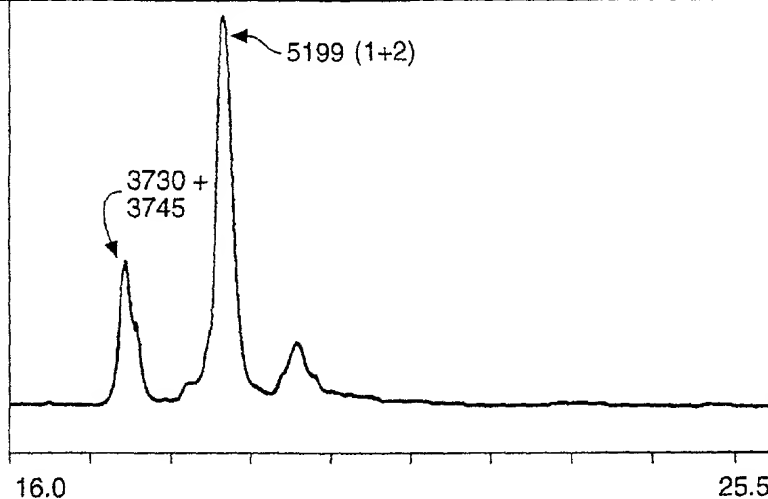


FIG._10A

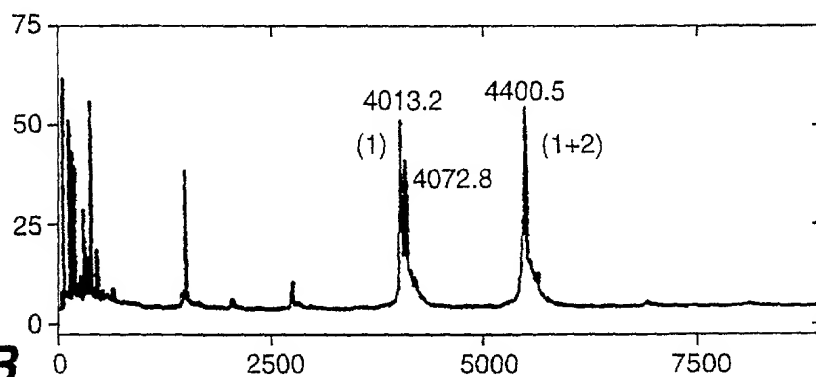


FIG._10B

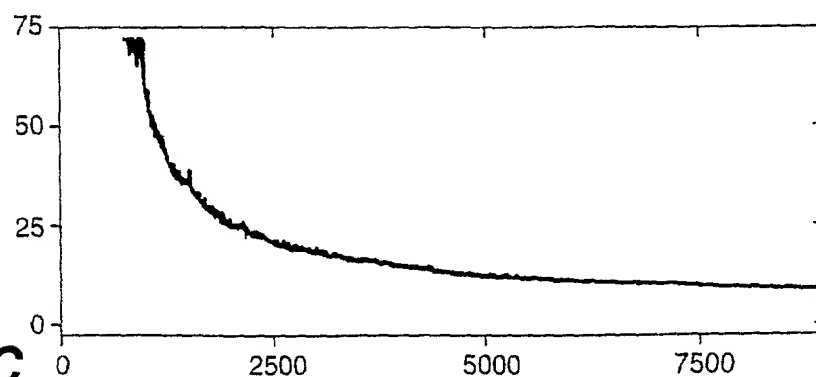


FIG._10C

Polymer-Supported
Ligation on ISCO

FIG. 11

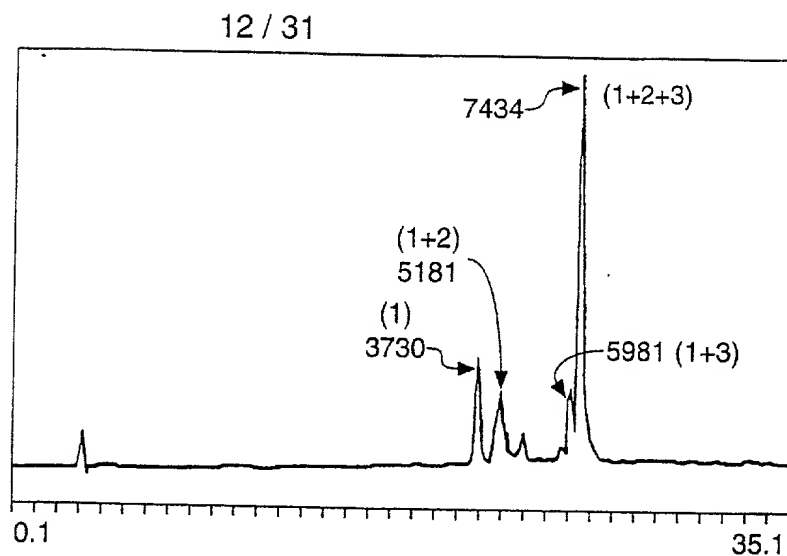


FIG. 12A

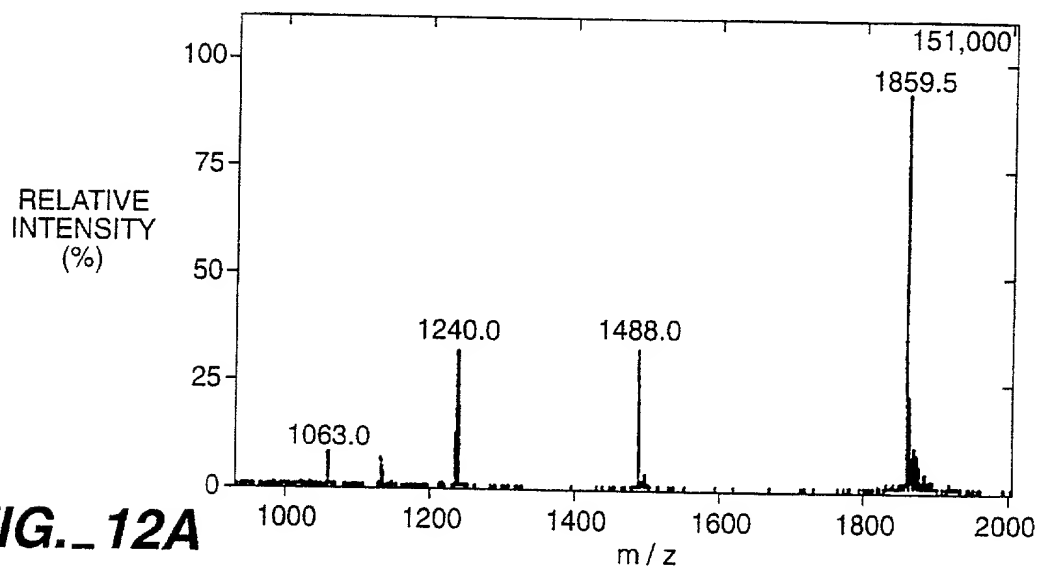
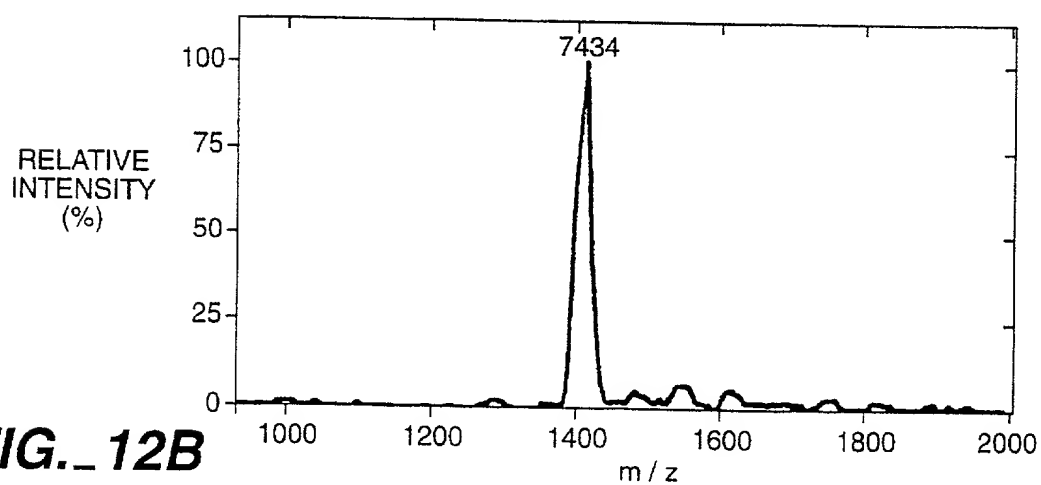


FIG. 12B



Polymer-Supported
Ligation on
PEGA

FIG._13

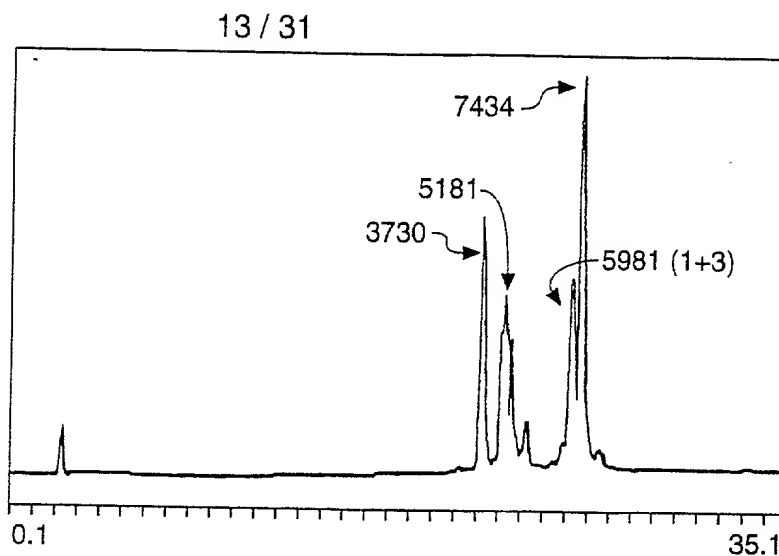


FIG._14A

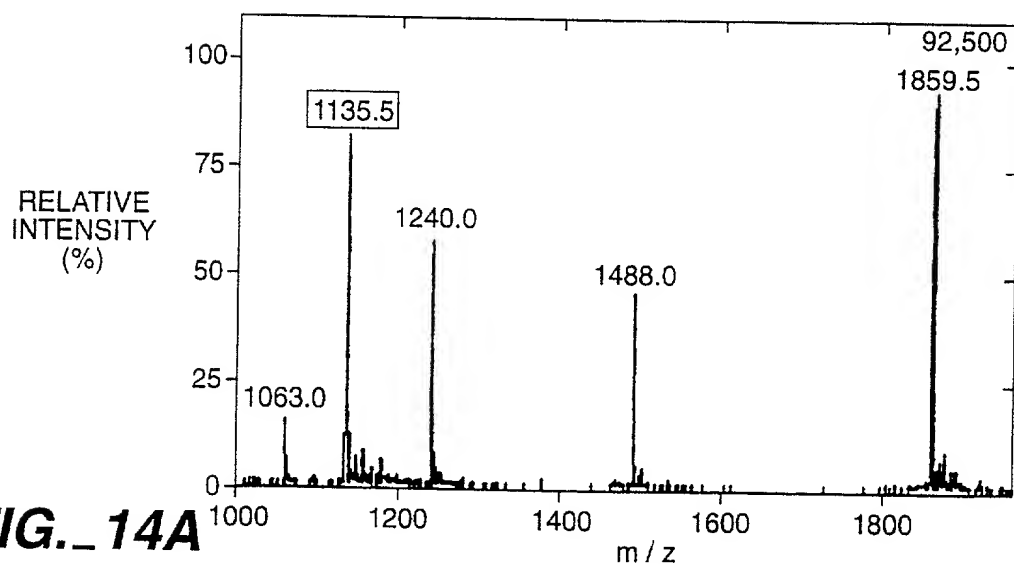
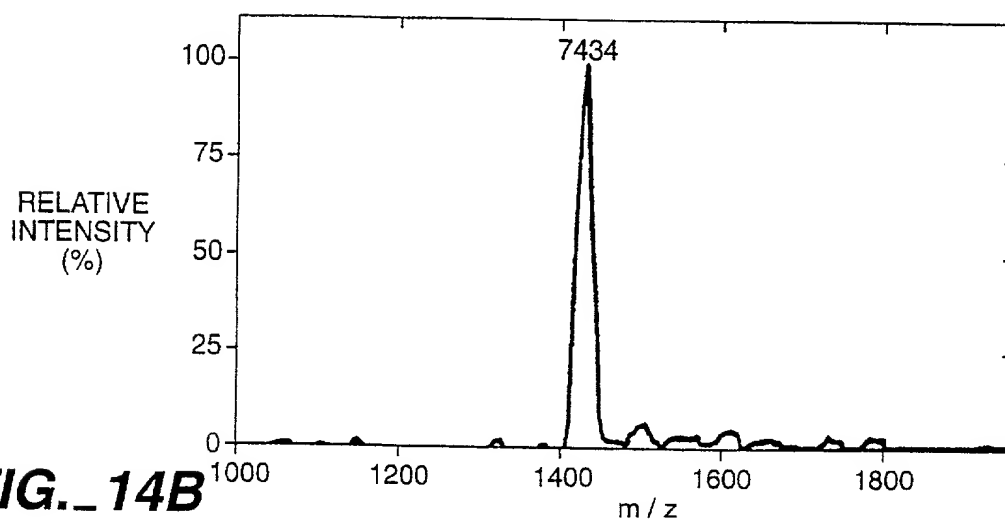


FIG._14B



On Resin
Purification

FIG._ 15A

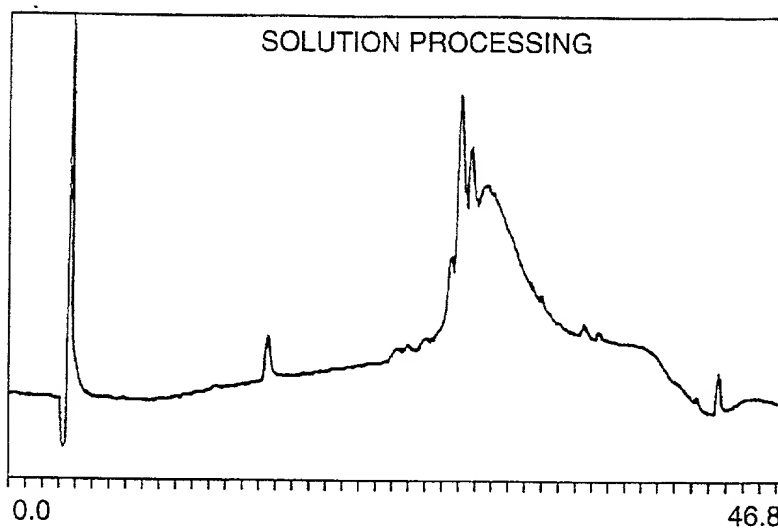


FIG._ 15B

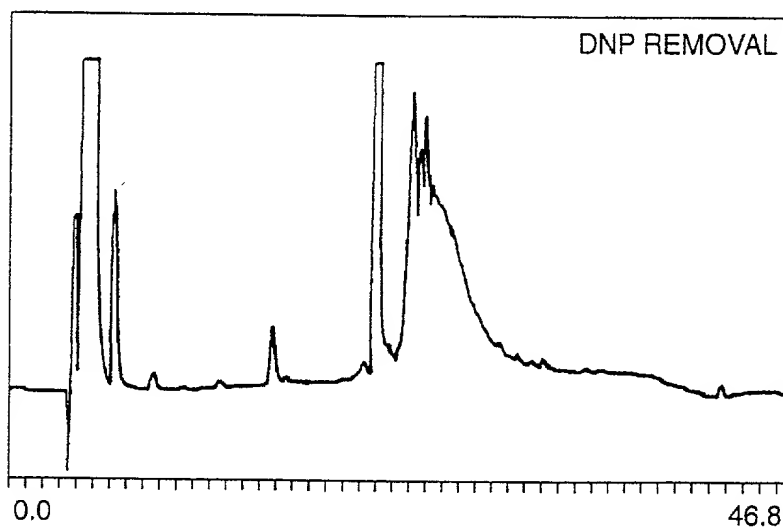
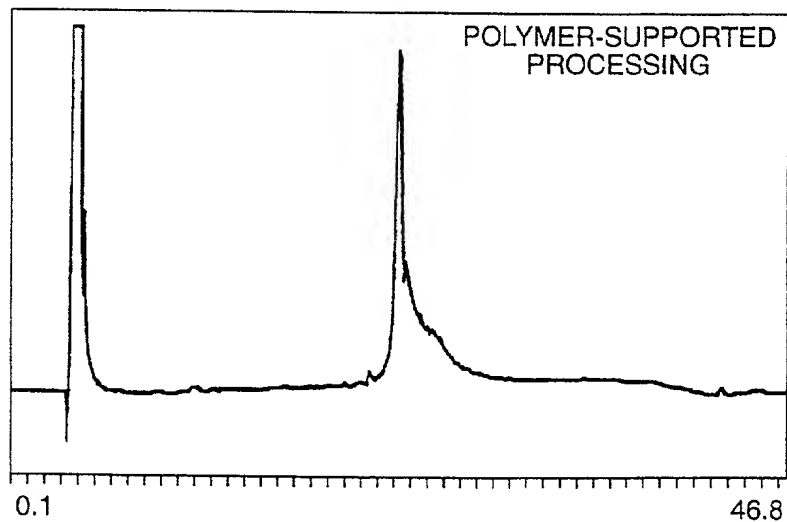


FIG._ 15C



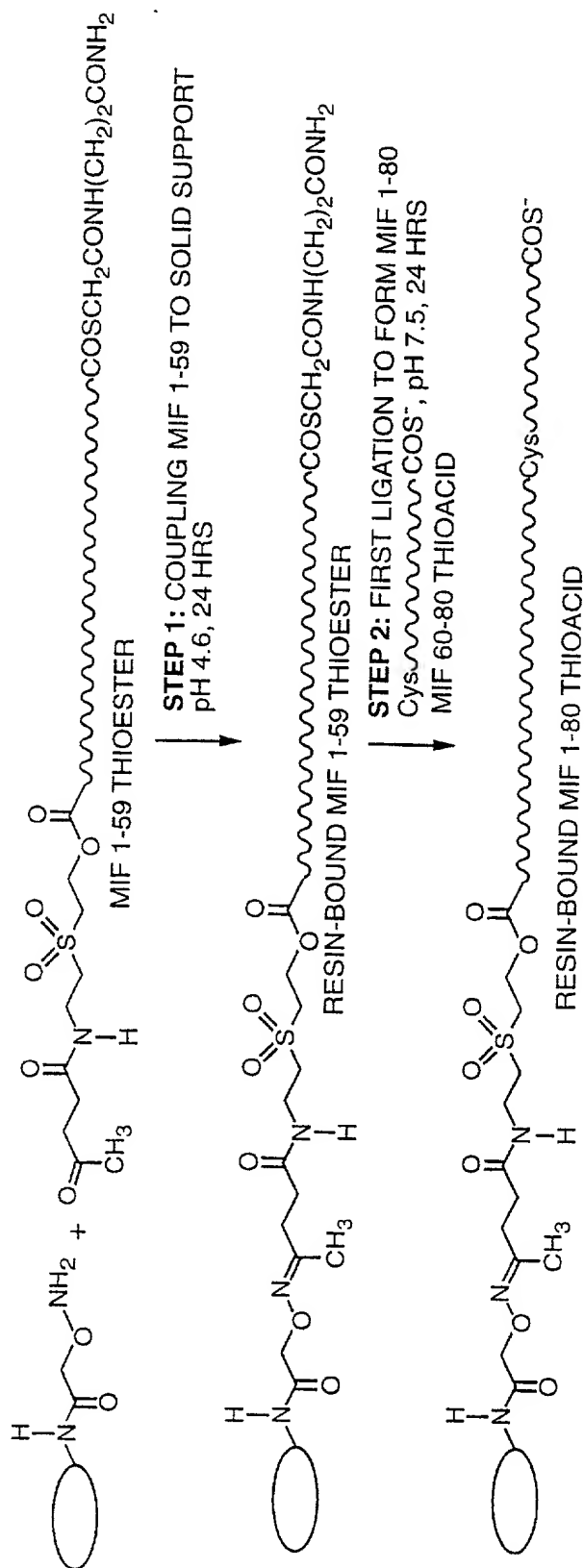


FIG. 16A

Synthesis of MIF by Solid Phase Native Ligations

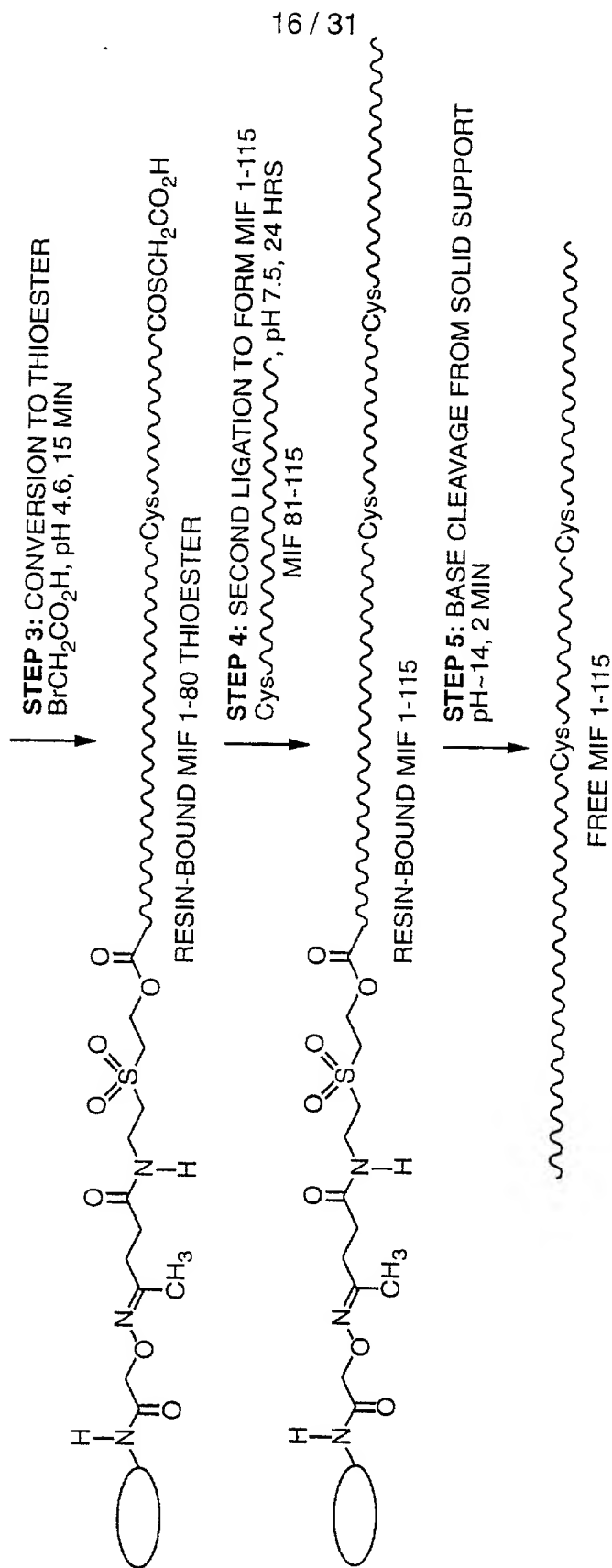
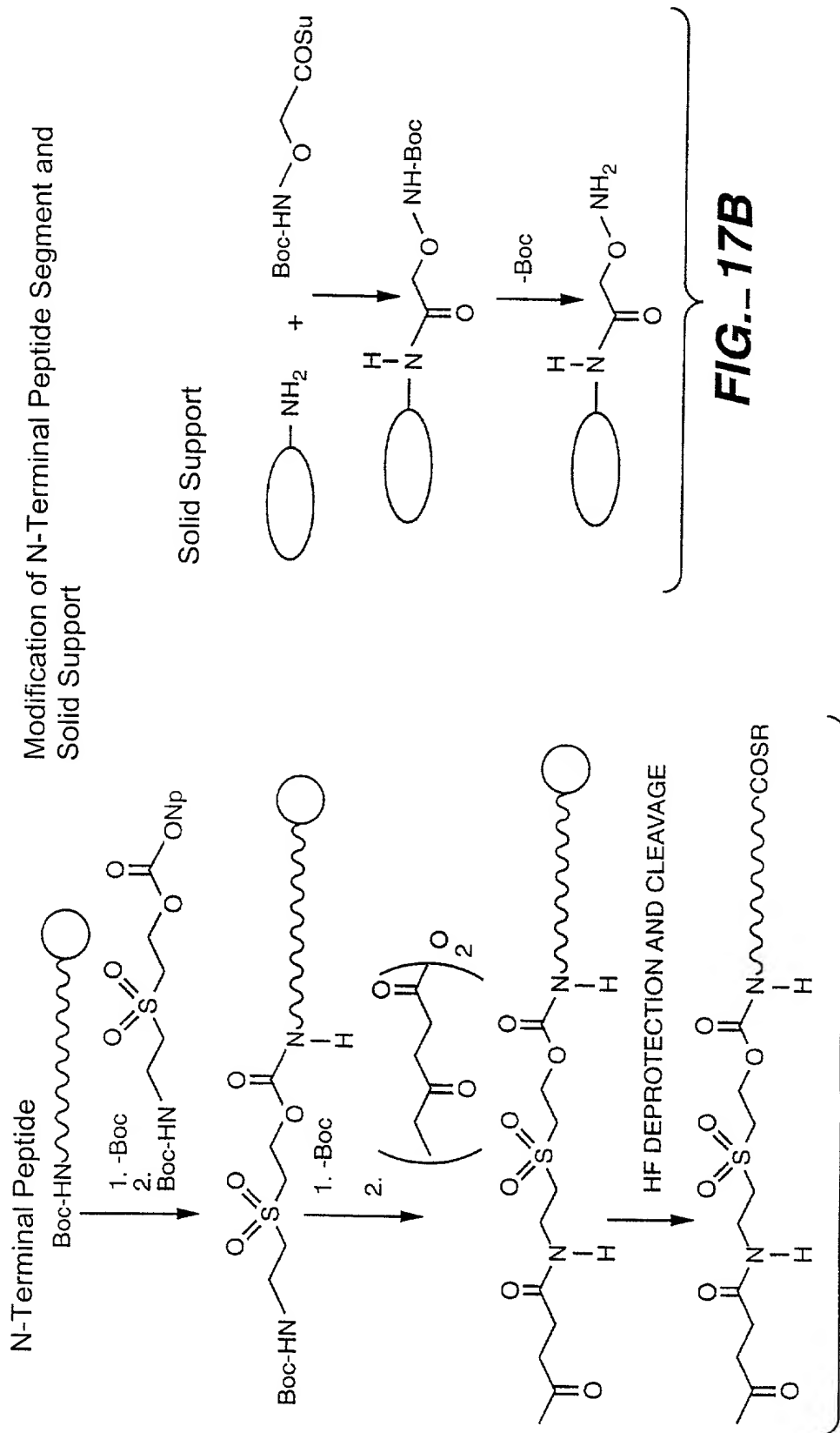
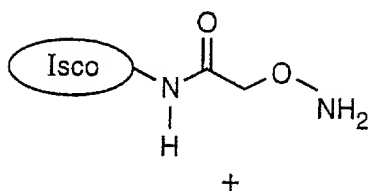


FIG. 16B



Coupling of MIF⁺
1-59 to Solid
Support

18 / 31



+

KETONE - MSC HANDLE - MET¹ - MIF 2 - 58 - Leu⁵⁹ - SAc - βAla - CO₂H

#1

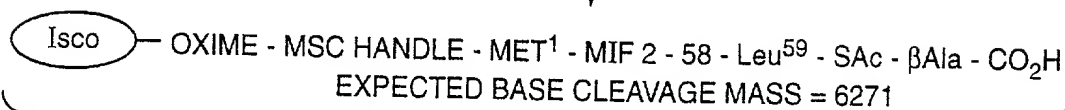


FIG._18A

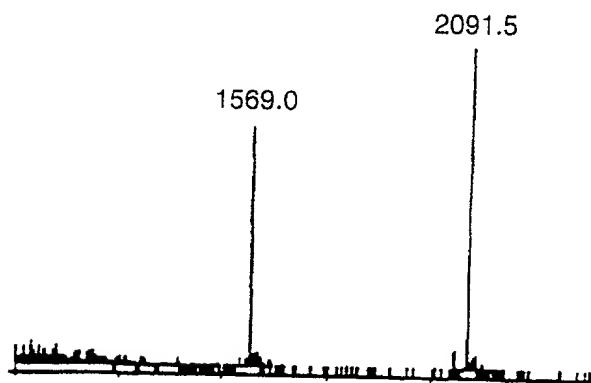


FIG._18C

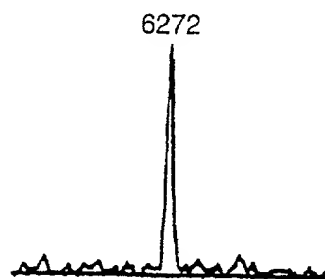
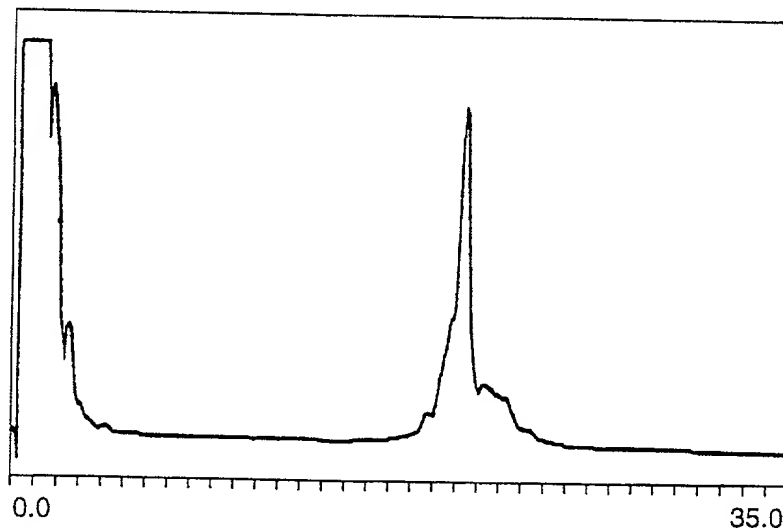


FIG._18D

FIG._18B



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Ligation to form MIF 1-80

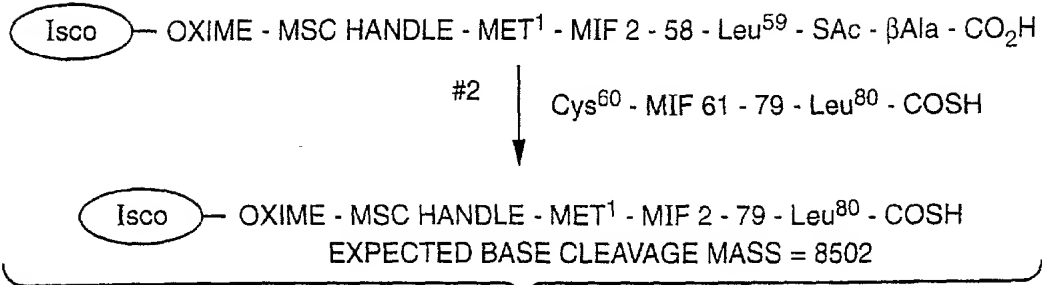


FIG._19A

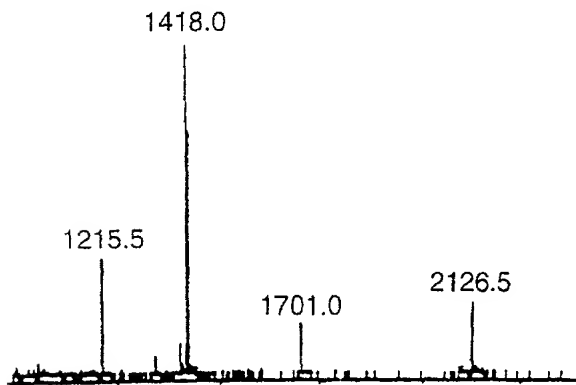


FIG._19C

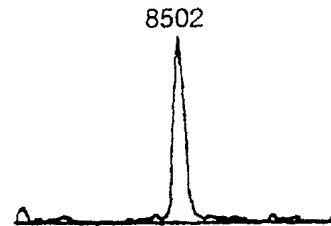


FIG._19D

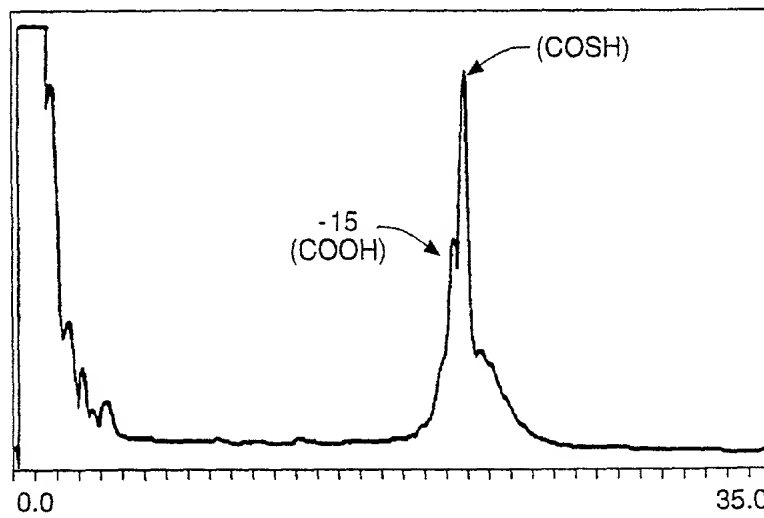


FIG._19B

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Ligation to form MIF 1-115

Isco

OXIME - MSC HANDLE - MET¹ - MIF 2 - 79 - Leu⁸⁰ - COSAc

#4

Cys⁸¹ - MIF 82 - 114 - Ala¹¹⁵ - CO₂H

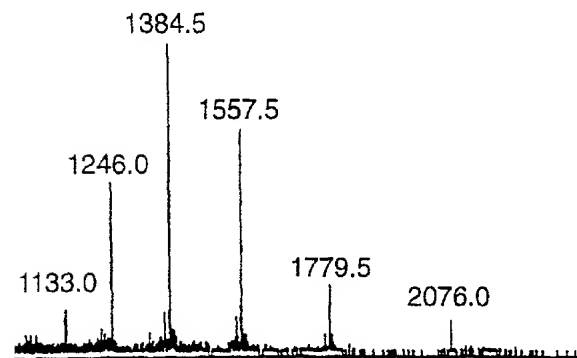
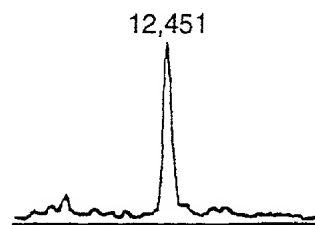
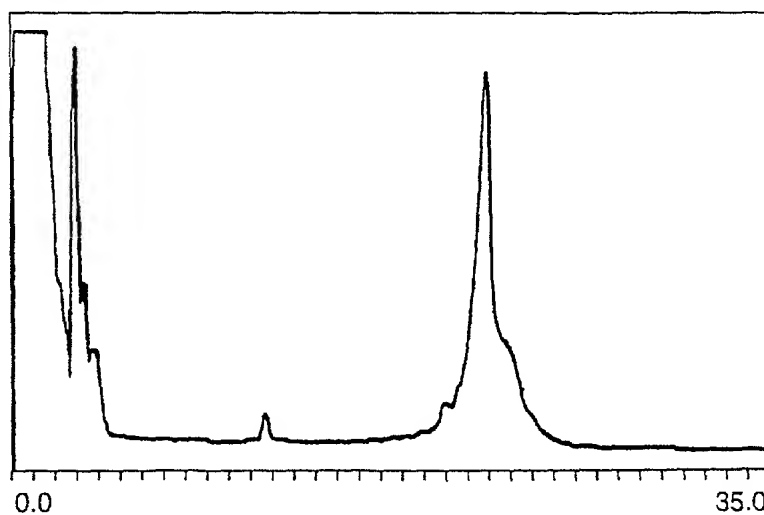
6M Gu•HCl, 0.1, 0.1 M Na Pi, 0.5% THIOPHENOL

0.15 M METHIONINE, pH 7.5

Isco

OXIME - MSC HANDLE - MET¹ - MIF 2 - 114 - Ala¹¹⁵ - CO₂H

EXPECTED BASE CLEAVAGE MASS = 12450

FIG._20A**FIG._20C****FIG._20D****FIG._20B**

Solid Phase Chemical Ligations in the C- to N-terminal Direction

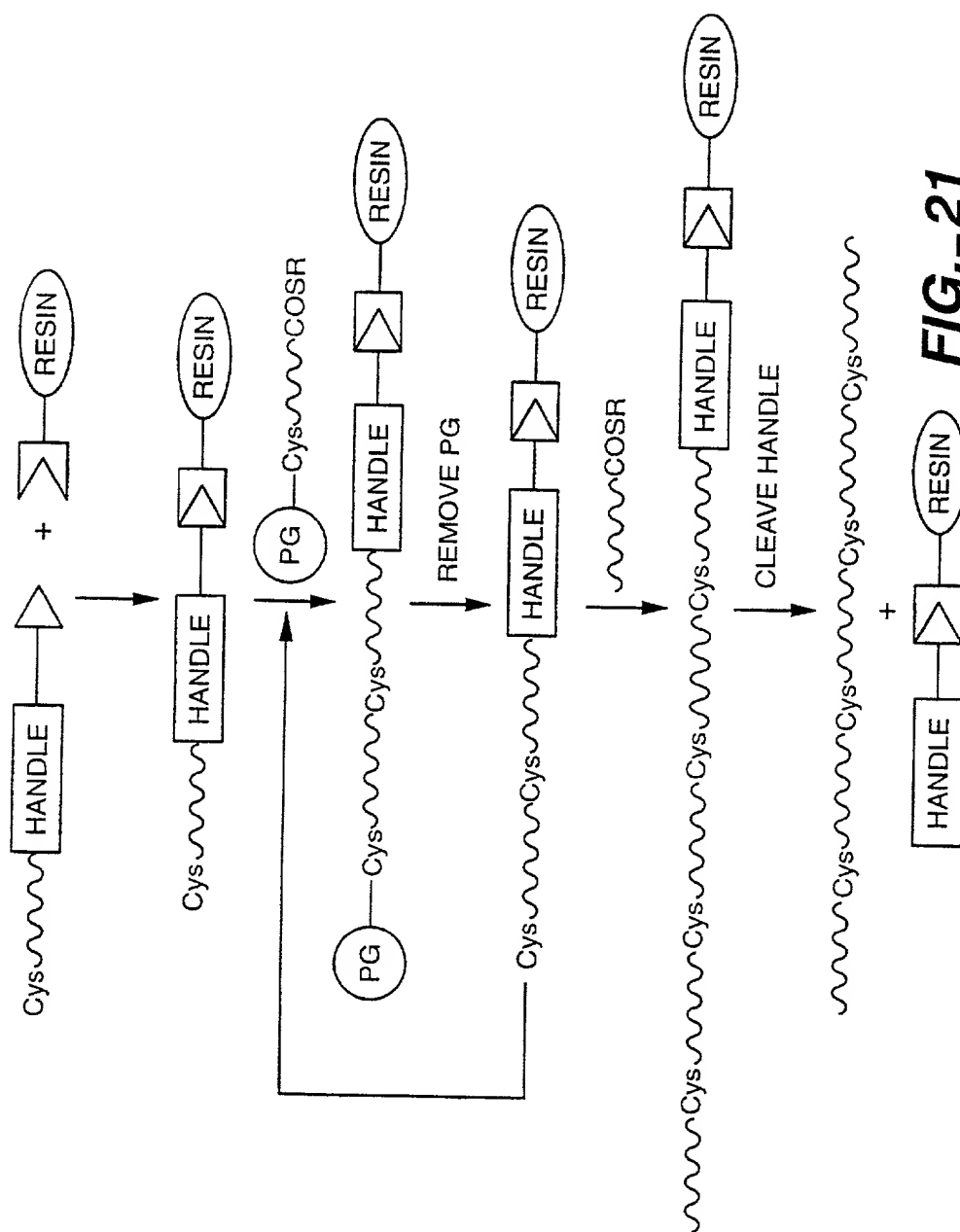
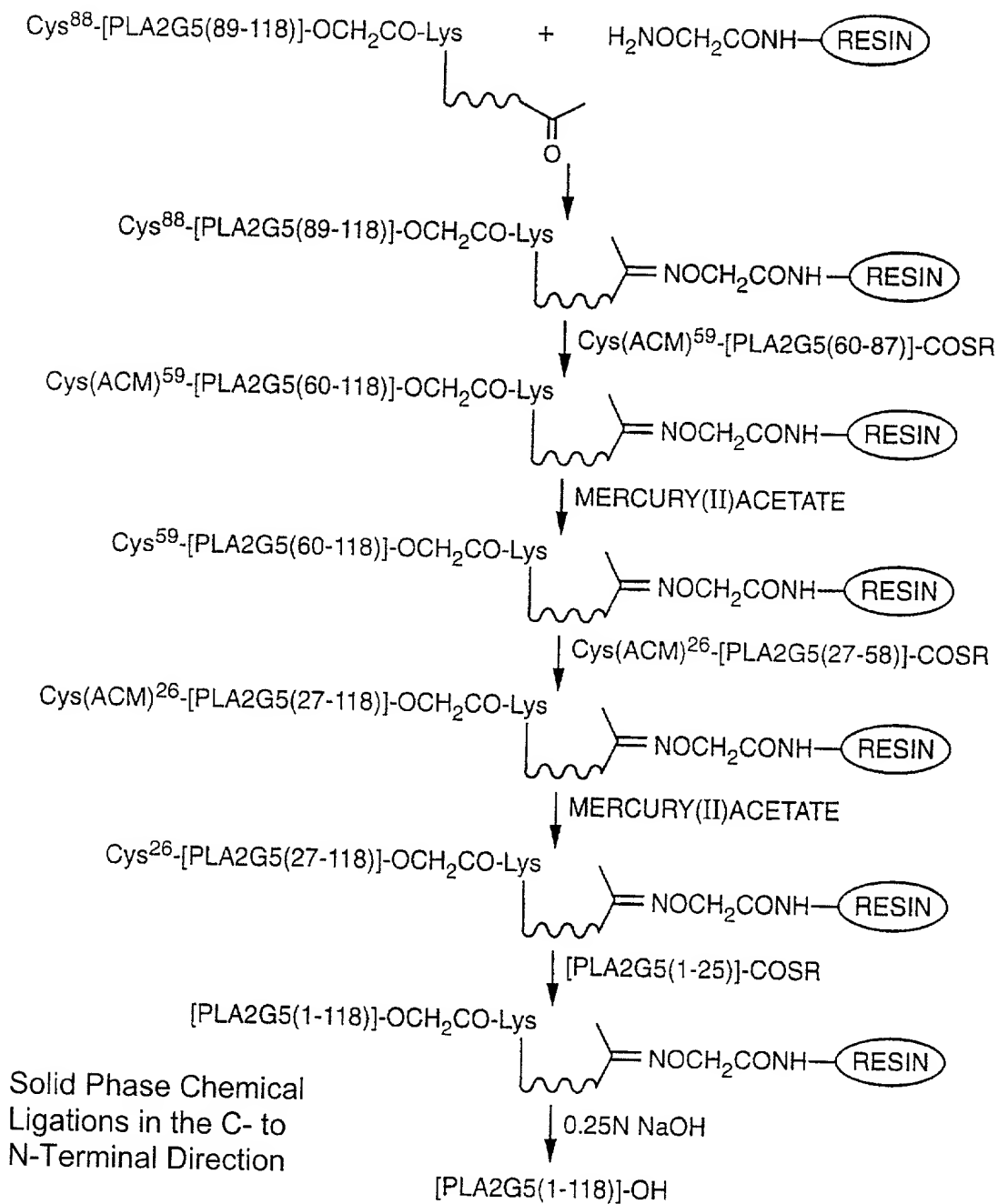


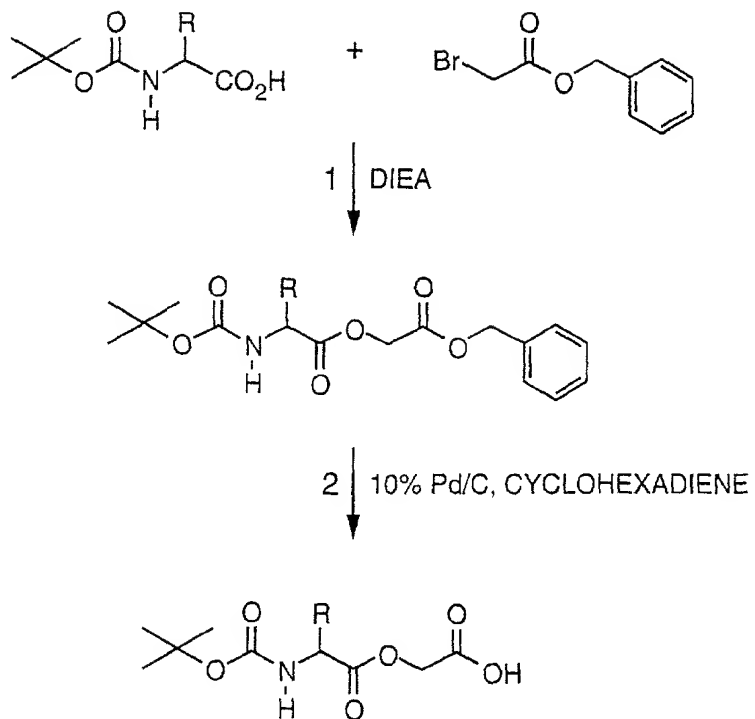
FIG. 21



Solid Phase Chemical
 Ligations in the C- to
 N-Terminal Direction

Synthesis of
 Phospholipase A2,
 Group 5 (PLA2G5)

FIG. 22

**FIG. 23**

Synthesis of Cam ester derivative

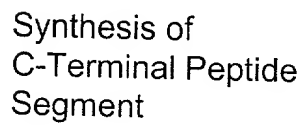


FIG. 24

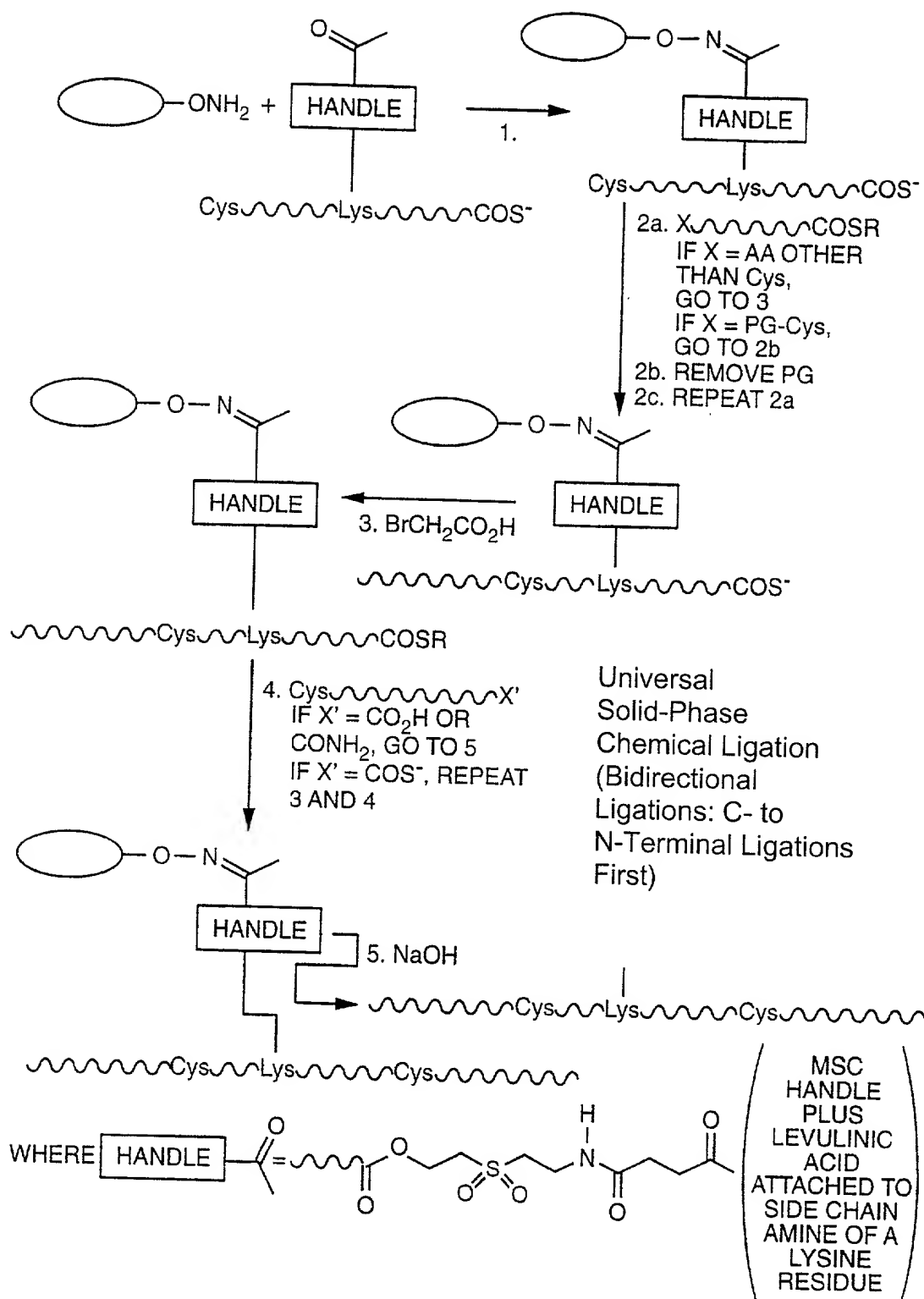


FIG. 25A

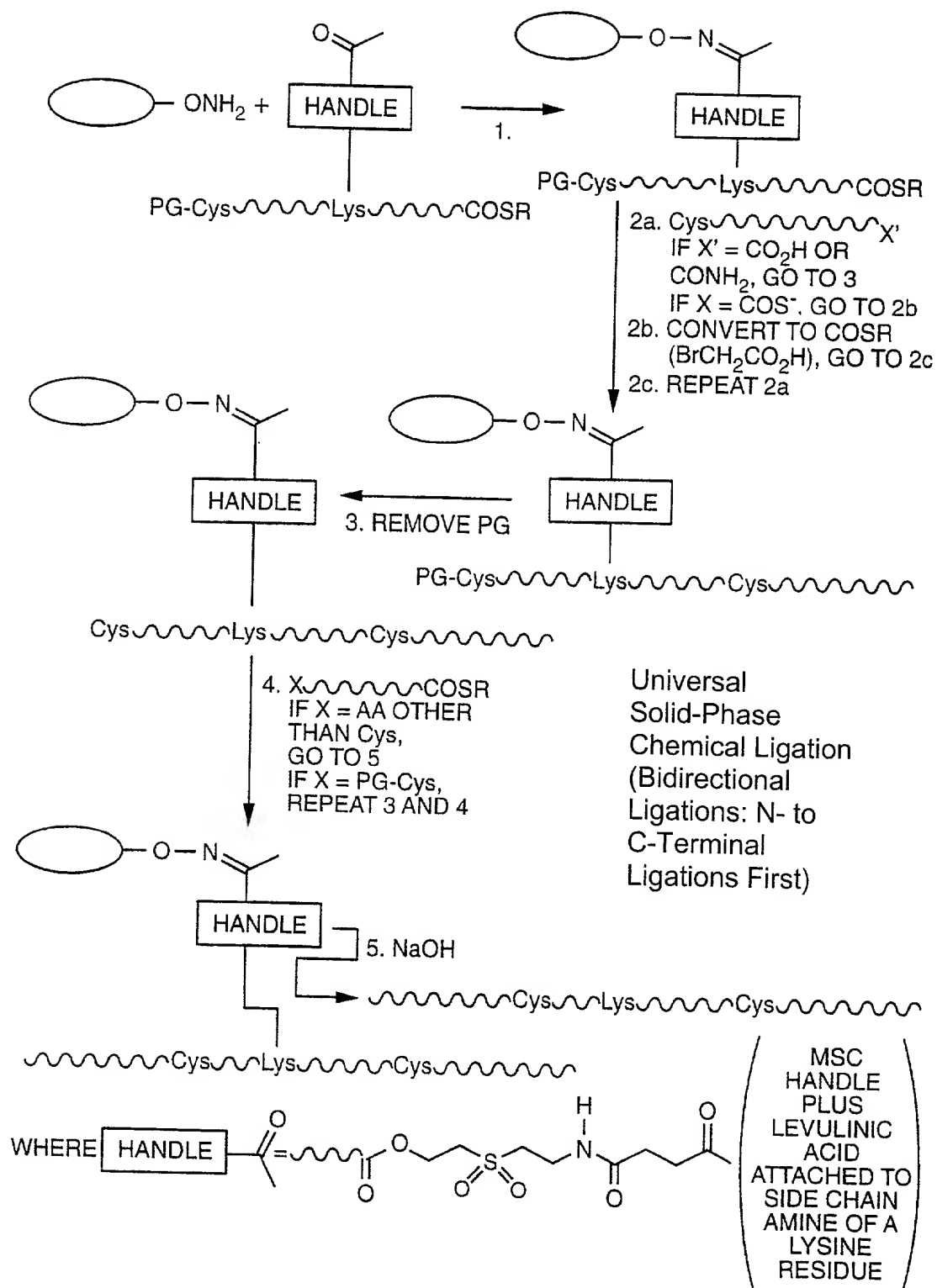


FIG. 25B

Synthesis of Modified Peptide Segment for Universal Solid Phase Chemical Ligation

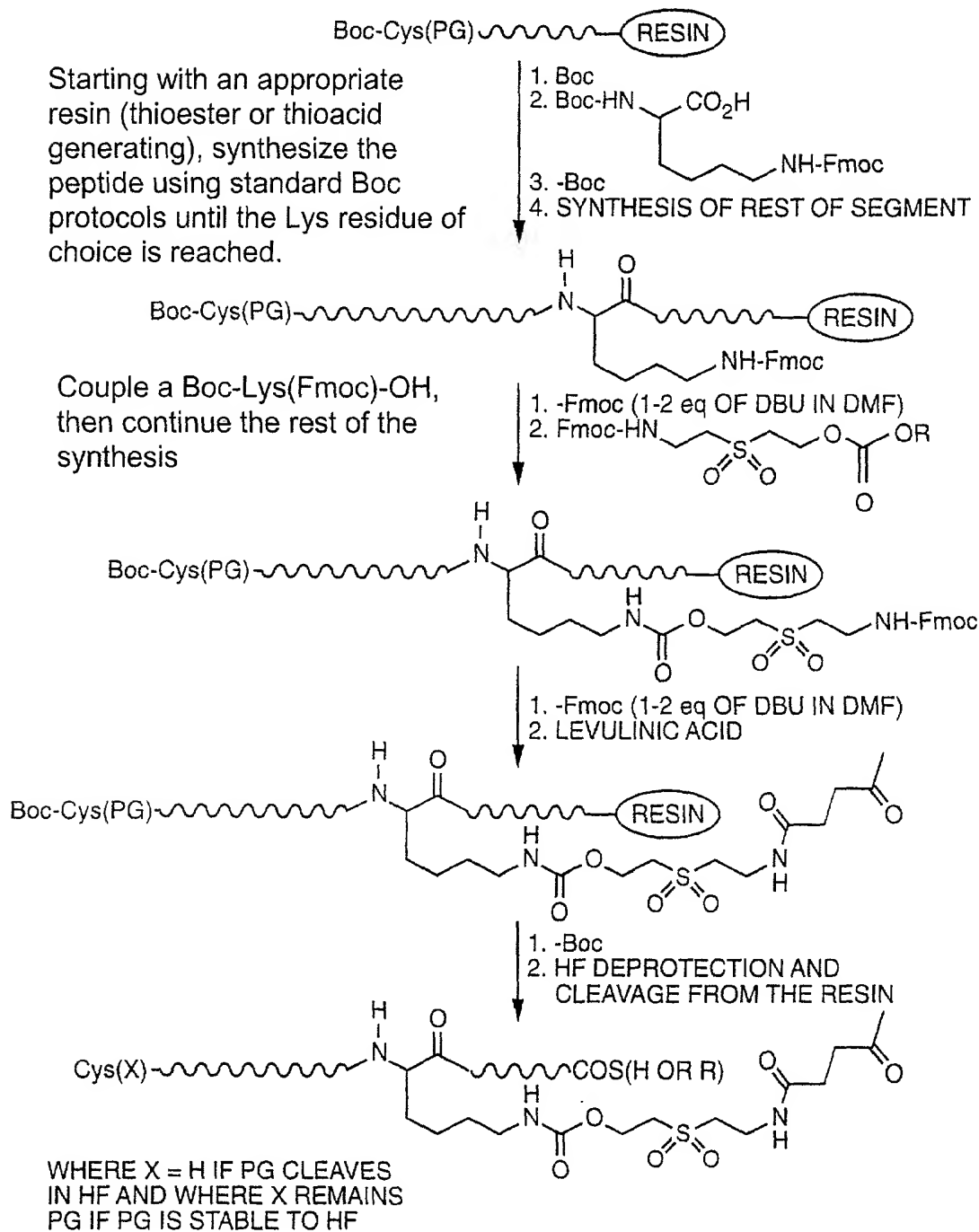
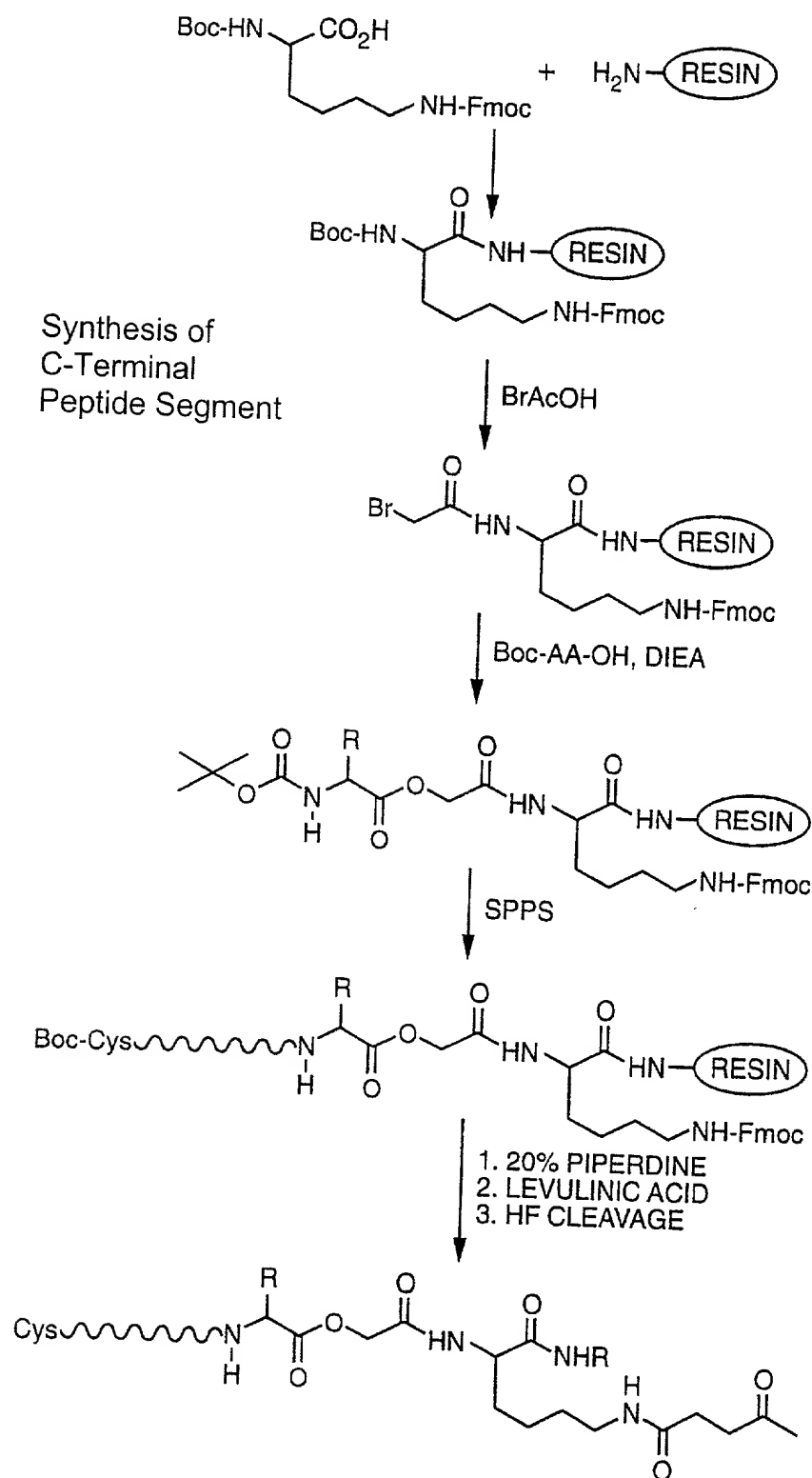


FIG..25C

**FIG. 27**

ALTKYGFYGCYGRLEEKGCADRKNILA
1 10 19 27

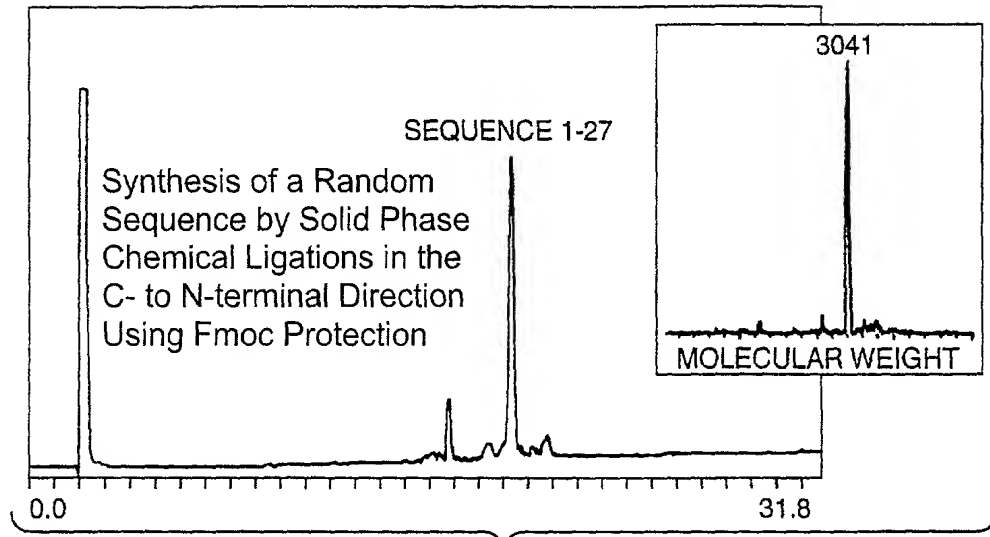


FIG._28

ALTKYGFYGCYGRLEEKGCADRKNILA
1 10 19 27

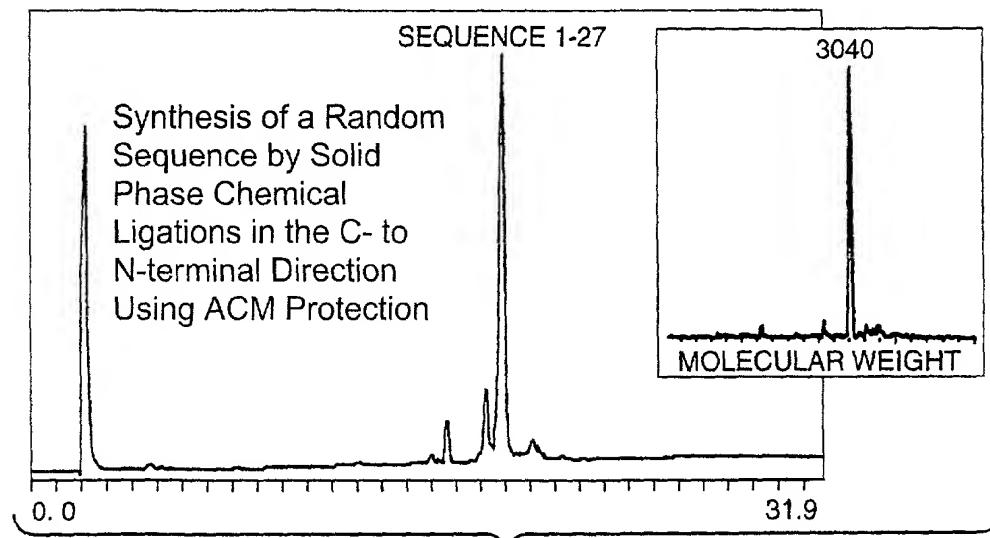
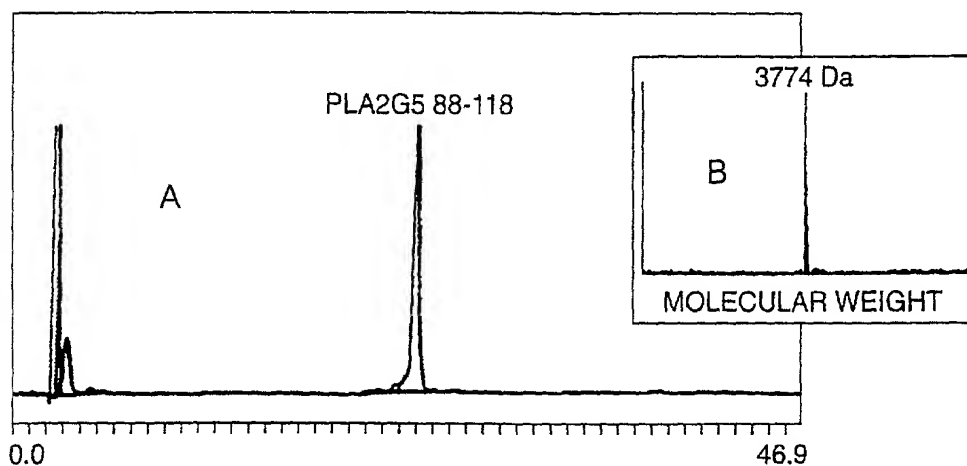


FIG._29

1 26 59
 GLLDLKSMIEKVTGKNALTNYGFG**CYCGWGGRGTPKDGT**DWCCWAHDHCYGRLEEKGC
 NIRTQSYKYRFAWGVVTCEPGPFCHVNL**CACDRKLVYCLKRN**LRSYN**PQYQYFPN**ILCS
 88 118



1 26 59
 GLLDLKSMIEKVTGKNALTNYGFG**CYCGWGGRGTPKDGT**DWCCWAHDHCYGRLEEKGC
 NIRTQSYKYRFAWGVVTCEPGPFCHVNL**CACDRKLVYCLKRN**LRSYN**PQYQYFPN**ILCS
 88 118

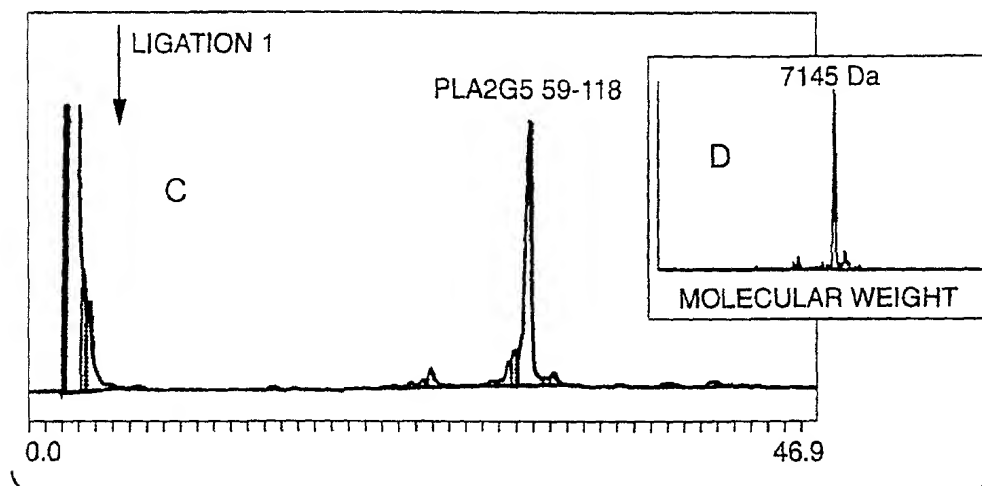
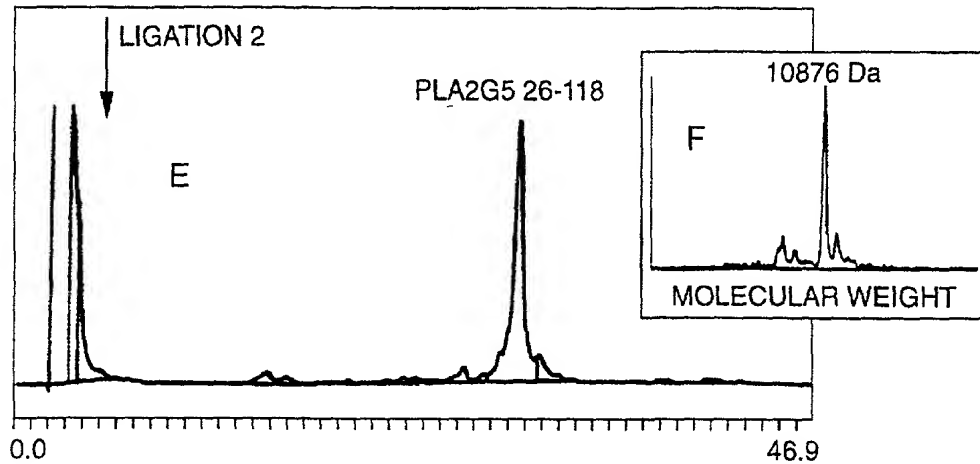


FIG. 30

1 26 59
 GLLDLKSMIEKVTGKNALTNYGFG**CYCGWGGRGTPKDGT**DWCCWAHDHCYGRLEEKGC
 NIRTQSYKYRFAWGVVTCEPGPFCHVNL**CACDRKLVYCLKRNLRSYNPQYQYFPN**ILCS
 88 118



1 26 59
 GLLDLKSMIEKVTGKNALTNYGFG**CYCGWGGRGTPKDGT**DWCCWAHDHCYGRLEEKGC
 NIRTQSYKYRFAWGVVTCEPGPFCHVNL**CACDRKLVYCLKRNLRSYNPQYQYFPN**ILCS
 88 118

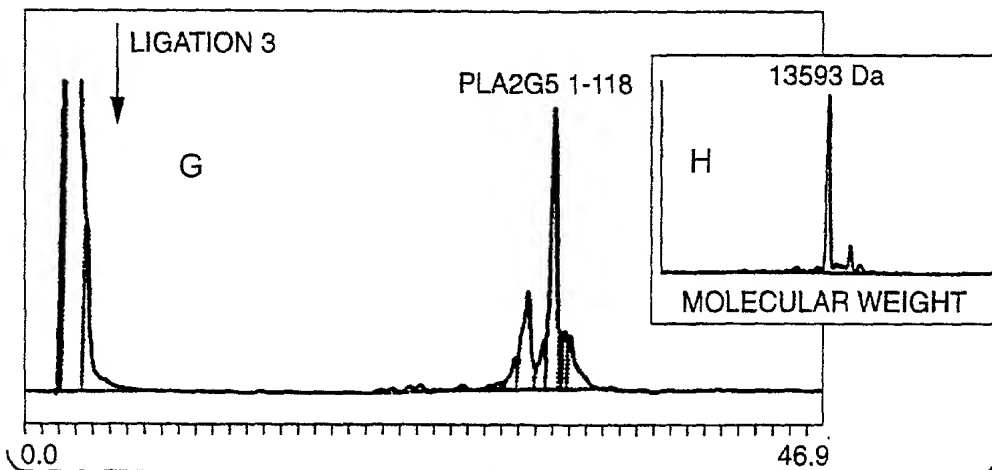


FIG. 30